METHODS FOR ASSESSING CELL SURFACE GLYCOSYLATION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The application is a National Stage application under 35 U.S.C. § 371 of International Application No. PCT/US2018/027666, filed on Apr. 13, 2018, which claims the benefit of priority to U.S. provisional patent applications 62/485,897, filed Apr. 14, 2017, entitled "METHODS FOR ASSESSING CELL SURFACE GLYCOSYLATION" and U.S. provisional application No. 62/515,515, filed Jun. 5, 2017, entitled "METHODS FOR ASSESSING CELL SURFACE GLYCOSYLATION," the contents of each of which are incorporated by reference in their entirety.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled 735042010800SeqList.TXT, created Oct. 10, 2019, which is 37,300 bytes in size. The information in the electronic format of the Sequence Listing is incorporated by reference in its entirety.

FIELD

[0003] Provided herein are methods for assessing cell surface glycans, e.g., N-glycans, by assessing a sample of released surface glycans, and determining the presence, absence, or level of glycans present in the sample. Also provided are methods of assaying and/or evaluating a cell composition by assessing the cell surface glycan profile of the cell composition and comparing the profile to a reference sample. Methods for manufacturing and/or culturing a plurality of cell compositions having consistent surface glycan expression with low variability are also provided.

BACKGROUND

[0004] Glycans are among the principal components of a cell. Nearly all human membrane proteins, as well as numerous intracellular proteins, are co- and post-translationally modified by the covalent addition of glycans. In particular, N-glycans are post-translational modifications to proteins that can have far reaching impact to structure and function. At a cellular level, glycosylation has been implicated in cell signaling, adhesion, homing properties and other functional activities. There exists a need in the art for additional methods to measure and identify glycans, e.g., N-glycans, that are expressed on the surface of cells.

SUMMARY

[0005] Provided herein are methods for assessing cell surface glycans, the methods comprising: (a) incubating a test composition comprising a plurality of cells under conditions to release one or more glycans from the surface of cells in the test composition, wherein a sample comprising one or more cell surface glycans is generated; and (b) determining the presence, absence, identity and/or level of glycans present in the sample, thereby assessing the cell surface glycan profile of the sample.

[0006] Also provided herein are methods for assessing cell surface glycans, the methods comprising determining the presence, absence, identity and/or level of glycans present in a sample, thereby assessing the cell surface glycan profile of the sample, wherein the sample comprises one or more glycans released from the surface of cells present in a test composition comprising a plurality of cells after incubation of the test composition under conditions to release the one or more glycans.

[0007] In some embodiments of any of the provided methods, the glycans are N-glycans. In particular embodiments of any of the provided methods, cells in the test cell composition comprise whole or intact cells. In some embodiments of any of the provided methods, the cells are live cells. In certain embodiments of any of the provided methods, the test cell composition is not homogenized or sonicated prior to the incubation; and/or the test cell composition is not incubated with a protease prior to the incubation, optionally wherein the protease is trypsin; and/or the cells in the test cell composition, prior to or during the incubation, are not contacted with an agent to extract one or more cell surface or membrane proteins, optionally wherein the agent is a detergent or protease, optionally trypsin; and/or less than 10% of the cells are lysed and/or ruptured during the incubation.

[0008] In particular embodiments of any of the provided methods, the test cell composition comprises no more than 5×10^6 cells. In some embodiments of any of the provided methods, the test cell composition comprises between 1×10^6 cells and 5×10^6 cells, inclusive. In certain embodiments of any of the provided methods, the test cell composition comprises a concentration of no more than 1×10^8 cells/mL. In particular embodiments of any of the provided methods, the test cell composition comprises a concentration of between 1×10^5 cells/mL and 1×10^8 cells/mL, inclusive, between 1×10^6 cells/mL and 5×10^7 cells/mL, inclusive, or between 5×10^6 cells/mL and 2.5×10^7 cells/mL, inclusive. In some embodiments of any of the provided methods, the incubation is carried out in the presence of an N-glycosidase. In certain embodiments of any of the provided methods, the N-glycosidase is a peptide N-glycosidase (PNGase) F. In particular embodiments of any of the provided methods, the PNGase F is recombinant.

[0009] In some embodiments of any of the provided methods, the one or more glycans are one or more N-glycans, and wherein the method comprises: (i) incubating between 1×10^6 and 5×10^6 cells from the test composition with a recombinant PNGase F under conditions to release the one or more N-glycans from the surface of the cells of the test composition; (ii) labeling the one or more N-glycans with a detectable label, optionally a fluorescent label; and (iii) determining the presence, absence, or level of the labeled N-glycans, thereby assessing the cell surface glycan profile of the sample.

[0010] In certain embodiments of any of the provided methods, the test cell composition comprises about 1×10^6 to 2.5×10^6 cells. In particular embodiments of any of the provided methods, the test cell composition comprises a concentration of between 1×10^6 cells/mL and 5×10^7 cells/mL. In some embodiments of any of the provided methods, the PNGase F comprises a PGNase F of *Flavobacterium meningosepticum*, or a portion or mutant thereof that is enzymatically active. In certain embodiments of any of the provided methods, the PNGase F comprises the amino acid sequence set forth in SEQ ID NO: 1 or a portion or mutant thereof that is enzymatically active, or an amino acid